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ROLE OF SOME BIOLOGICALLY ACTIVE SUBSTANCES IN THE MECHANISM OF BLISTER FORMATION*

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The etiology and pathogenesis of vesiculobullous eruptions have been investigated actively for the past two decades, but the mechanisms involved in the formation of a blister are not yet adequately understood. Attempts have been made to produce blisters by artificial means such as heat (1, 2) cantharidin (3–7), proteolytic enzymes (8, 9), and several other chemicals (10). Several workers (11–13) studied proteins, electrolytes and cells in the blister fluid and suggested that these elements are mainly derived from blood. In a recent study, Kandhari and Pasricha (14) obtained similar results, and concluded that in all likelihood the significant process involved is that of increased capillary permeability. Substances like 5-hydroxytryptamine (5-HT, serotonin), histamine and acetylcholine are commonly known to be associated with processes such as vasodilatation, increased capillary permeability and exudation. Experiments were, therefore, designed to detect, by bioassay, these substances in the blister fluid, and to attempt to determine their role in bulla formation.

MATERIAL AND METHODS

Material for study was comprised of 11 cases of pemphigus, 8 cases of thermal burns, 4 cases each of erythema multiforme bullosum and epidermolysis bullosa and 1 case each of herpes zoster, dermatitis venenata and insect bite. The clinical diagnosis in cases of pemphigus, erythema multiforme bullosum and epidermolysis bullosa was confirmed by histopathological examination of skin biopsy specimens.

In 10 human subjects artificial blisters were induced by applying 0.5% cantharidin ointment in wool fat base. The quantity applied varied between 50–100 mg, spread on about 1 square inch area of the forearm skin, and left for 6 to 8 hours. The fluid which accumulated 18 to 24 hours later into the blister cavity was collected and used for assay.

Blister fluid was collected from intact blisters, centrifuged to separate away cellular debris, and stored at 0° C for further use unless processed immediately.

5-HT, histamine and acetylcholine were extracted from blister fluid by standard procedures, and were then estimated on isolated tissues by the bioassay methods. Appropriate doses of known and unknown samples were used in the bioassay procedure and concentration of the drug in the unknown sample was calculated by plotting a log-dose response graph (15). Specificity of the bioassay procedure was checked by using appropriate antagonists of the test substances, 5-HT, histamine and acetylcholine.

5-HT was extracted from blister fluid by the method of Amin *et al.* (16) and was tested on fundus of the rat's stomach (17).

Histamine was extracted from the blister fluid by the method of Parratt and West (18) and tested on the isolated guinea pig ileum (19).

For acetylcholine assay, equal volume of eserine sulfate solution (10 mg/liter) was immediately added to the blister fluid and the pH of the mixture was brought to 4 by adding dilute HCl (20). The fluid was then tested on the isolated abdominal rectus muscle of the frog (20).

Throughout this report the values of 5-HT and histamine represent the weight of the bases and not of their salts.

Further 0.5 μg of 5-HT and 175 μg of histamine each in 0.1 ml of normal saline were injected intradermally into the skin of 10 subjects with pemphigus and 10 normal human volunteers, and the response was noted for up to 24 hours.

RESULTS

5-HT was estimated in all the 40 cases included in this study, histamine in 29 cases and acetylcholine in only 4 cases (Table I).

The highest concentration of 5-HT was obtained in the blister fluid of epidermolysis bullosa subject. 5-HT was generally higher in blister fluids of cases of burns and epidermolysis bullosa. It was absent in 7 cantharidin-induced blisters, whereas it could always be detected in the fluid of spontaneous blisters irrespective of their etiology.

The highest concentration of histamine was found in erythema multiforme bullosum. It was absent in about half of the cases of pem-

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TABLE I

Levels of 5-hydroxytryptamine, histamine and acetylcholine in various vesiculo-bullous dermatoses
The values have been expressed in Nanograms/ml (1/1000th of a microgram).

Biologically active substances		Pemphigus	Epidermolysis bullosa	Erythema multiforme bullosum	Thermal burns	Cantharidin induced blisters in normal volunteers
5-HT	Total No. of cases	11	4	4	8	10
	Mean value	10	78	29	92	3
	Range	(2–23)	(11–223)	(17–46)	(36–197)	(0–18)
Histamine	Total No. of cases	6	3	3	8	6
	Mean value	31	Nil	86	13	Nil
	Range	(0–76)	—	(0–250)	(0–30)	—
Acetylcholine	Total No. of cases	3	—	—	—	1
	Mean value	Nil	—	—	—	Nil
	Range	—	—	—	—	—

phigus and burns (Fig. 1). It was not detected in cases of epidermolysis bullosa and cantharidin-induced blisters.

Acetylcholine was not found in any of the 3 cases of pemphigus and one of the cantharidin-induced blisters.

Levels of 5-HT and histamine in each insect bite, herpes zoster and dermatitis venenata cases were 54, 8, 57 nanogram/ml and nil, nil, 38 nanogram/ml respectively.

Eight normal subjects and 10 pemphigus patients showed marked erythema at the site of 5-HT intradermal injection. Intradermal histamine injection produced pronounced erythema and wheal in all the normal and pemphigus cases. Blister formation was not observed in any of the experiments.

DISCUSSION

Studies on protein and electrolyte content of blister fluid and serum strongly suggest that serum is the main source of blister fluid and that the process of formation of blister fluid involves increase in capillary permeability (14). In looking for the substance(s) which increase capillary permeability it was necessary to evaluate the role played by 5-HT, histamine and acetylcholine. No studies on the levels of 5-HT and acetylcholine have been reported to-date.

In the present study, 5-HT was found in all the samples but the levels showed wide variation of individual values. No correlation could be established between the levels of 5-HT in the blister fluid and presence and severity of clinical signs and symptoms like itching, burning, pain, erythema, pulse rate and blood pressure in the individual patients. Since many of our pemphigus patients were on corticosteroid therapy at the time of collection of blister fluid, an attempt was also made to see if corticosteroids were responsible for the wide variation in the levels of 5-HT. However, no such correlation could be obtained between the 5-HT values and dose-duration of corticosteroid therapy.

Histamine was believed to be present in blister fluid of pemphigus cases (21). Burbach (22) could detect histamine in 1 of the 4 samples of pemphigus blister fluid. In the present study histamine was detected in 3 of the 6 samples of pemphigus blister fluid. These 3 reports together suggest that histamine may not necessarily be present in pemphigus blister fluid.

5-HT, histamine and acetylcholine do not seem to play any significant role in the formation of blisters for the following reasons:

5-HT and histamine levels in blister fluids showed marked variation and were unrelated to clinical signs and symptoms. Acetylcholine could not be detected in any of the four samples. Intradermal injections of comparatively large doses of 5-HT and histamine did not

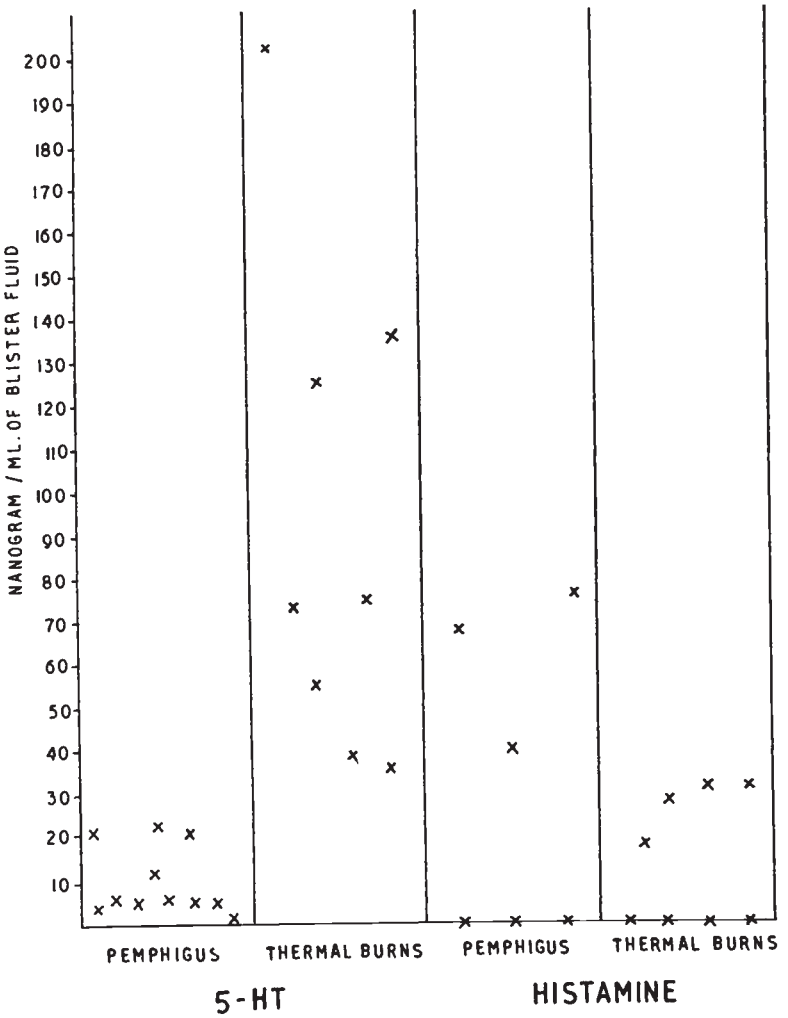

FIG. 1. Scattergram showing levels of 5-HT and histamine in individual cases of pemphigus and thermal burns.

induce blisters even in patients with pemphigus.

SUMMARY

Levels of 5-HT, histamine and acetylcholine were studied in the blister fluid in a group of 40 patients with vesiculo-bullous conditions (pemphigus, thermal burns, erythema multiforme bullosum, epidermolysis bullosa, herpes zoster, dermatitis venenata, insect bite and cantharidin induced blisters). 5-HT was found in all instances, histamine in about 50% of the subjects, and acetylcholine in none. Levels of these substances in each disease varied very widely and were unrelated to clinical signs and symptoms attributable to them. Intradermal injections of large doses of 5-HT and histamine did not produce blister in patients with pemphigus and in normal volunteers. These substances do not appear to play any significant role in the formation of blisters.

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